HybStation and Hyb4 Frequently Asked Questions

Digilab FAQ

1. Why do I need a HybStation?

ybridization is critical to the whole process of microarray analysis. Currently most researchers perform hybridizations under a coverslip in a humid chamber. The movement of the labeled probe (target) is by diffusion only and this is a very slow and in-efficient process. The HybStation agitates the hybridization mix and maximizes the movement of the probe.

2. How many slides can I Hybridize?

The HybStation is designed to automatically hybridize and wash up to 12 slides simultaneously, the slides are arranged in pairs on 6 modules.
The Hyb4 is designed to automatically hybridize and wash up to 4 slides arranged in pairs on 2 modules

3. Do I need to place slides in all positions?

No you may hybridize a single slide on a single module, this does require a blank slide.

4. Can the modules be individually controlled?

Yes, modules can run at different temperatures and have different wash protocols associated to them.

5. What is the maximum number of wash solutions?

The HybStation and Hyb4 can have up to 5 different wash reservoirs.

6. What volume is the hybridization chamber for each slide?

The volume is between 100 and 110 µl.

7. What is the hybridization area?

The usable hybridization area on each slide is 21.4 x 61 mm on the 21mm Lids

8. Do I need more labeled RNA sample?

No, a coverslip hybridization may require as little as 15 µl for the hybridization, with the HybStation the same probe is diluted to a final volume of 100-110 µl. This has little or no effect on sensitivity because of the agitation feature of the HybStation

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9. How does the agitation feature work?

There is a small inlet tube at the top left side of each slide compartment. There is a valve in this tube, which opens and closes repeatedly during a hybridization run. This creates positive and negative pressure cycling that results in circulation of the buffer over the slide during hybridization. Hybridization fluid is not, however, removed from the slide at any time during this process.

10. What is the temperature range of the HybStation and Hyb4?

Currently the range is 4°C to 100°C. The maximum ramp rate between 100°C and 10°C below ambient is 1°C/second.

11. What hybridization buffer is compatible?

We routinely test hybridization buffers as they enter the market and have a TechNote in buffer compatibility. Generally many buffer give acceptable results, however more viscous buffers, such as ULTRAhyb™ from Ambion, requires higher temperatures for the initial medium stringency wash.

12. How is probe added to the slides?

This is simply performed using a standard micropipette and disposable tip through an injection port on the surface of the module.

13. How much RNA/cDNA probe is used per slide?

The amount of RNA/cDNA probe required is dependent on the labeling technique used. For direct incorporation methods, we recommend labeling 50 µg of total RNA. In cases where RNA is limited, indirect methods may be used which require lower amounts of total RNA, 1 to 10 µg total RNA depending in method.

14. What post-hybridization buffers should I use?

Post-hybridization buffers are similar to conventional filter hybridizations, a range of different SSC solutions, ranging from 2 x SSC to 0.05 x SSC.

15. Are the slides dried automatically?

We recommend leaving the slides wet in a post wash buffer, containing medium stringency SSC and no SDS and drying the slides in a centrifuge.

16. Why are there two waste bottles on the HybStation?

To allow segregation of waste, if required. Apart from that, both bottles are identical.

17. What side of a frosted slide is best to hybridize to?

To ensure a good seal around the entire slide, we recommend that the slides be printed on the non-frosted side or have a bar code placed on the non-printed side.

18. Can I use less than a full volume in the hybridization chamber?

We recommend filling the chamber up to the first diffusion chamber.

19. Can I use Poly-L-Lysine slides?

Yes, however slides that have a thick coating can result in high background and stripping of the coating. An approved protocol for producing Poly-L-Lysine slide can be found at:

http://baygenomics.ucsf.edu/protocols/comp4/Poly-L-Lysine_slide_sop.pdf

20. Do you have recommended protocols?

Yes, we have numerous application notes for different types of arrays and labeling techniques. A good basic protocol can be found at:

http://baygenomics.ucsf.edu/protocols/comp4/Hybridization_SOP.pdf

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